Ecology of *Megalodiscus temperatus* (Digenea: Paramphistomatidae) in Red-spotted Newts, *Notophthalmus v. viridescens,* from West Virginia

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ABSTRACT: One hundred twenty-four red-spotted newts (55 females and 69 males) were collected from a marsh in western West Virginia throughout 1995. The large intestines of these salamanders were examined for the amphistome *Megalodiscus temperatus*. Prevalence of infection at 63.6% for female newts and 47.8% for males was not significantly different ($\chi^2 = 2.48$, $P > 0.05$). Mean intensities of 5.6 and 3.5 were recorded for female and male newts, respectively, and the difference between those means was significantly different (Mann–Whitney $U = 780$, $P < 0.05$). Numbers of amphistomes were positively correlated with host weight. Mean adult worm length was negatively correlated with numbers of individuals in a host. *Megalodiscus temperatus* adults were smallest in the May/June collection period and attained their maximum lengths in October and December. May/June appeared to be the primary recruitment period because of the high proportion of juveniles and small size of adult forms.

KEY WORDS: density dependence, *Megalodiscus temperatus, Notophthalmus,* red-spotted newt, West Virginia.

A considerable amount of information on prevalence and mean intensity of *Megalodiscus temperatus* (Stafford, 1905) Harwood, 1932, infections in newts exists today primarily because of the efforts of Mann (1932) and Rankin (1937) in North Carolina, Rankin (1945) in Massachusetts, Russell (1951) in Virginia, Fischthal (1955) in New York, Jackson and Beaudoin (1967) in Pennsylvania, and Price and Buttner (1982) in Illinois. Still, *M. temperatus* infections by host sex are seldom reported, and some investigators have presented data gathered over ambiguously defined or brief time frames (Rankin, 1945; Fischthal, 1955; Jackson and Beaudoin, 1967; Price and Buttner, 1982), leaving questions about the composition of the amphistome population at different times of the year. As a result, one of the goals of this study was to segregate newts by sex and provide information on *M. temperatus* prevalences and mean intensities by host sex for each of 5 defined collection periods throughout 1995. Because collections of *M. temperatus* individuals were sufficiently large in each of the collection periods, we expanded the scope of our investigation to address questions pertaining to 1) the numbers of amphistomes as a function of host size, 2) the size of these worms as a function of their numbers in a host (i.e., a crowding or density-dependent effect), 3) the life cycle stages of worms present by collection period, and 4) the size of adult worms in each of 3 defined reproductive

categories by period of collection. The first of these 4 questions was prompted by Rankin's (1937, p. 219) generalization that the number of parasites per host increased with size and age of hosts. The second question arose in response to the observation (without supporting data) by Fischthal (1955) that smaller sexually mature forms of *M. temperatus* were obtained when crowding occurred in the large intestine. The second question took on added meaning with the realization that density-dependent mechanisms are considered of central importance in stabilizing the population growth of parasitic organisms (Anderson, 1978; Anderson and May, 1978) at least in part by suppressing parasite survival or fecundity (Anderson and Gordon, 1982). Density dependence in parasite survival and fecundity might arise as a result of 2 distinct phenomena: intraspecific competition for finite resources such as food or space and/or the generation of immune responses (Anderson, 1978; Keymer, 1982). The general view that fitness of individual worms is reduced within hosts as worm density increases was reiterated by Goater (1992). *Megalodiscus temperatus* appears to be a good model to examine the intraspecific competition phenomenon because this species is confined to the newt's large intestine, a relatively small region of the gastrointestinal tract that could present, presumably, food and space constraints. The third and fourth questions were prompted by the realization that our collections could reveal

Table 1. Numbers of *Megalodiscus temperatus* juveniles and adults collected from newts, specifying by collection period those adults used for length and reproductive category determinations.

Collection period	No. worms*							
	n_C	n_X	n_O	n_J	n_A	n_{Az}	n_{Aa}	n_{Ab}
Feb/Mar	59	26	33	0	0/33	0/0	0/13	0/20
May/Jun	75	8	67	20	33/47	1/1	26/36	6/10
Aug	71	3	68	1	55/67	6/8	39/49	10/10
Oct	61	2	59	1	58/58	0/0	13/13	45/45
Dec	46	1	45	1	44/44	8/8	10/10	26/26
Total	312	40	272	23	190/249	15/17	88/121	87/111

* n_C = total number *M. temperatus* collected at necropsy (shown in Fig. 1); n_X = number lost or damaged; n_O = number observed ($n_C - n_X$); n_J = number of juveniles (not measured for length); n_A = number of observed adults ($n_O - n_J$). Numerator indicates number of adults suitable for length measurements and corresponds to collection period/All n_A values in Figure 2. Denominator indicates number of adults suitable for determining reproductive category. n_{Az} = nongravid adults; n_{Aa} = gravid adults with 1–25 eggs; n_{Ab} = gravid adults with >25 eggs. Relative proportions of adults in each of these reproductive categories by collection period are depicted in Figure 3, and relative lengths are shown in Figure 4.

some new information about *M. temperatus* biology (e.g., period of recruitment, worm size at different times during the year, and relative fecundity levels of adults).

Megalodiscus temperatus is a common digenetic trematode of amphibians in the United States (Cheng, 1986). Eggs laid by adults reach the water via the hosts' feces and hatch into miracidia, which infect *Helisoma* spp. snails. Polyembryonic development within these molluscs results in the production of cercariae, which escape from the snail and encyst in the skin of larval and adult newts. There is no second intermediate host characteristic of so many digenetic trematode life cycles; adult newts ingest their sloughed skin (Morgan and Grierson, 1932) or cannibalize their larvae (Burton, 1977) to acquire infective metacercariae.

Materials and Methods

One hundred twenty-four newts (55 females and 69 males) were collected by hand or in funnel traps from Shoals Marsh, a permanently flooded marsh in Wayne County, West Virginia (38°19'45"N, 82°28'18"W) over 5 collection periods; February/March, May/June, August, October, and December. Each newt was sexed, weighed to the nearest 0.1 g, and then euthanized by pithing within 24 hr of capture. The large intestine was then removed, and all *M. temperatus* were counted and killed by fixation in 10% buffered formalin at room temperature (adults only) under slight coverslip pressure. All adults and a few metacercariae were stained in acid carmine, dehydrated in an ethanol series, cleared in xylene, and mounted in Permount®. Adult amphistomes were measured with a calibrated ocular micrometer. Voucher specimens of *M. temperatus* were deposited in the U.S. National Parasite Collection, Beltsville, Maryland: accession numbers 87276 (ju-

veniles), 87277 (nongravid adult), 87278 (gravid adult with 1–25 eggs), and 87279 (gravid adult with >25 eggs). Juveniles were characterized by the absence of reproductive organs and the presence of distinctly branching eyespots. Nongravid forms possessed testes and ovaries but lacked eggs. Eyespots were sometimes present in these adults but were less extensive than those seen in metacercariae. The 2 different categories of gravid adults were simply determined by egg counts.

Upon examining our total data set, 1 problem was immediately apparent; although we had collected 312 *M. temperatus* individuals, some were lost and thus their length measurements and reproductive status could not be determined. The problem was compounded by the fact that no attempt was made to prepare adult trematodes for measurements in the initial collection period of February/March, but the reproductive status could be determined for 33 of those adults. In other cases, length measurements could not be obtained because the trematodes had been damaged upon removal from their host (or improperly fixed after removal), yet their reproductive status could still be ascertained. To deal with these deficiencies in our data set, we constructed Table 1 to provide an accounting of numbers of juveniles and those adult worms in various reproductive categories suitable for body length measurements and reproductive status determination. Sample numbers for each reproductive category in Table 1 carry over into Figures 1–4.

Gender differences in prevalences were evaluated with a chi-square test. Because density data were not normally distributed, differences in mean intensities were analyzed using a nonparametric Mann–Whitney U-test. When data were normally distributed (e.g., host weights), t-tests were employed. F-tests were used in determining the significance of b-values for all regressions. Statistical analyses were performed according to Sokal and Rohlf (1995), and P-values <0.05 were considered significant for all tests. Prevalence and mean intensity of infection follow the definitions of Bush et al. (1997).
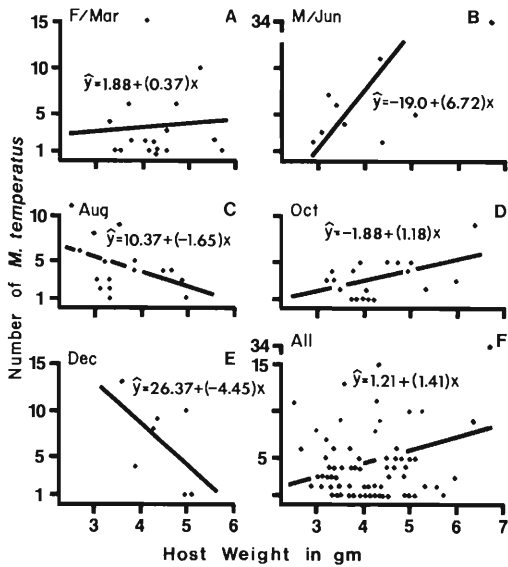
Figure 1. Scatterplots of the relationship between total *Megalodiscus temperatus* and host weight (sexes combined) by collection period (A–E) and for all periods combined (F). Relationships are significant (i.e., $b \neq 0$) for May/June, October, and all collection periods combined.

Figure 2. Scatterpolots depicting length of *Megalodiscus temperatus* individuals as a function of numbers present in a given host by collection period (A–D) and for all collection periods combined (E). Negative relationships for August and all collection periods combined are significant (i.e., $b \neq 0$). Each dot represents 1 measured individual, n_A = the number of adults suitable for measurement (see Table 1).

Results

Prevalences and mean intensities of infection

Prevalence of *M. temperatus* infection in female newts (63.6%) was not significantly different from that of males (47.8%) ($\chi^2 = 2.48$, $P = 0.139$) (Table 2). Based on pooled samples, there was an observable seasonal pattern in prevalence: 37.8% in February/March, rising steadily to 86.4% in October, and then declining to 35.0% in December (Table 2). Temporal change in prevalence appeared unrelated to host size. The highest (86.4%) and second lowest (37.8%) prevalence values were recorded in collection periods when infected hosts had mean comparable weights of 4.29 g and 4.31 g, respectively. In addition, the second highest prevalence (76.2%) was recorded in August, when mean infected host weight was the lowest (3.60 g).

Mean intensities of *M. temperatus* infection for female (5.6) and male (3.5) hosts were significantly different (Mann–Whitney $U = 780$, $P < 0.05$) (Table 2). Seasonal differences in mean intensity between female and male newts were not critically examined because of high sample variances associated with small sample sizes of 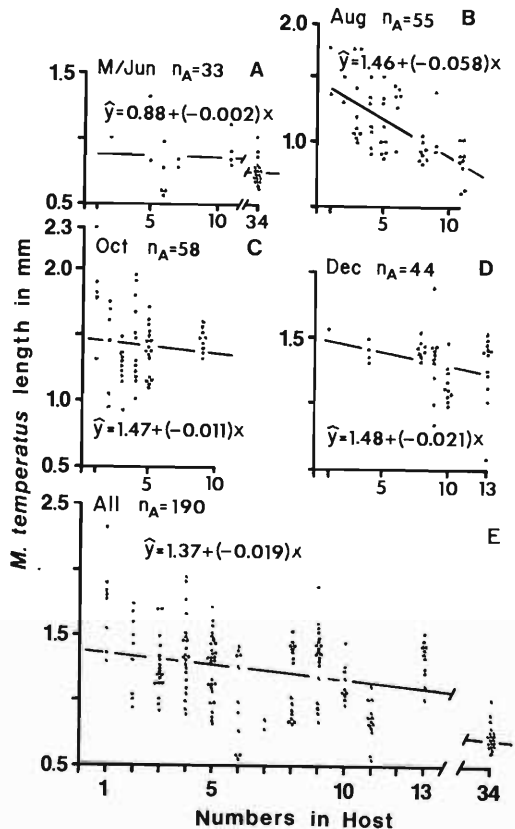1 or both infected host sexes in most collection periods (Table 2). Male newts infected by *M. temperatus* were heavier ($\bar{x} \pm$ SD = 4.25 $\pm$ 0.69 g) than infected females (3.99 $\pm$ 1.01 g), but this difference was not significant ($t_{0.05,66} = 1.23$; $P > 0.05$). There was no significant difference ($t_{0.05,53} = 0.495$; $P > 0.05$) in weights of infected females (3.99 $\pm$ 1.01 g) versus uninfected females (4.17 $\pm$ 1.69 g), but infected males (4.25 $\pm$ 1.69 g) were significantly heavier ($t_{0.05,67} = 3.84$; $P < 0.05$) than uninfected males (3.44 $\pm$ 1.02 g).

Relationships between parasite numbers and host size

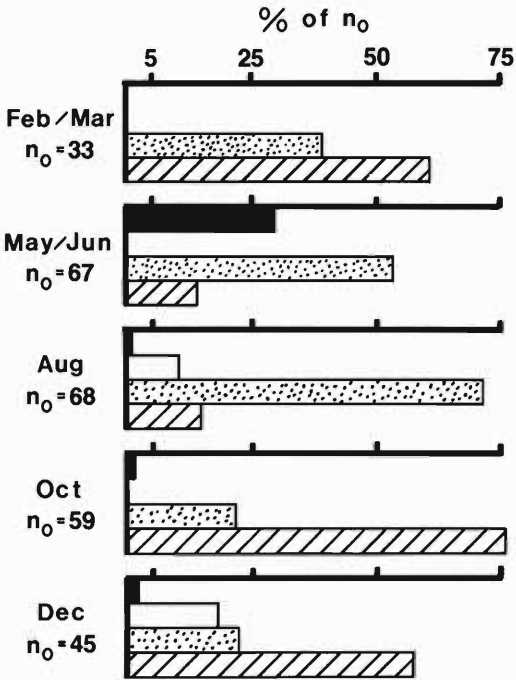Numbers of *M. temperatus* individuals as a function of host weight (worms from both sexes

Figure 3. Relative frequencies of *Megalodiscus temperatus* life cycle stages in red-spotted newts by collection period (see n_o, Table 1, for sample sizes). Solid bars = juveniles; open bars = nongravid adults; stippled bars = gravid adults with 1–25 eggs; cross-hatched bars = gravid adults with >25 eggs.
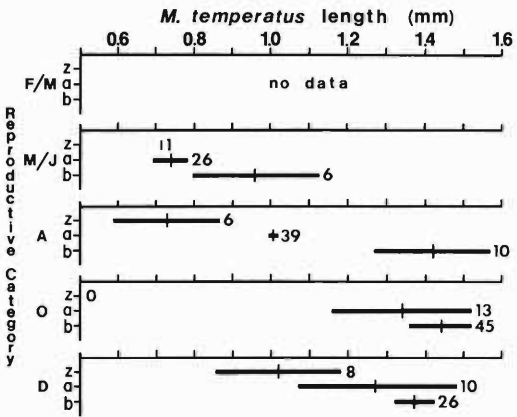

Figure 4. Mean lengths of adult *Megalodiscus temperatus* individuals by reproductive category and collection period. z = nongravid adults; a = gravid adults with 1–25 eggs; b = gravid adults with >25 eggs, respectively. Vertical lines = means; horizontal lines = 95% confidence limits around the means. Numbers to the right of confidence limits are the numbers of worms measured to calculate means and correspond to the collection period numerators in columns n_{Az}, n_{Aa}, and n_{Ab} of Table 1.

of infected newts combined) varied with collection period (Fig. 1A–E). This relationship was positive and significant in May/June ($b \neq 0$, $F_{0.05[1,7]} = 14.66$, $P < 0.05$) and October ($b \neq 0$, $F_{0.05[1,17]} = 5.91$, $P < 0.05$) (Fig. 1B, D). A positive relationship observed in February/March (b

= 0, $F_{0.05[1,15]} = 0.067$, $P > 0.05$) and negative relationships in August ($b = 0$, $F_{0.05[1,14]} = 3.34$, $P > 0.05$) and December ($b = 0$, $F_{0.05[1.5]}$, $P > 0.05$) were not significant (Fig. 1A, C, E). Overall (Fig. 1F), numbers of *M. temperatus* were positively and significantly correlated with host weight ($b \neq 0$, $F_{0.05[1,66]} = 4.52$, $P < 0.05$).

Density-dependent analyses

Lengths of those adult *M. temperatus* individuals suitably prepared for measurement (Table 1) were negatively correlated with total numbers of individuals present in a host for every collec-

Table 2. Prevalence and mean intensities for *Megalodiscus temperatus* infections in newts at Shoals Marsh by sex of host and collection period.

Collection period	Prevalence*			$\bar{x}$ intensity (SD)	
	Females	Males	Pooled	Females	Males
Feb/Mar	3/8 (37.5)	14/37 (37.8)	17/45 (37.8)	3.0 (2.6)	3.6 (4.4)
May/Jun	5/8 (62.5)	4/8 (50.0)	9/16 (56.3)	10.4 (13.4)	5.8 (3.8)
Aug	16/21 (76.2)	0/0	16/21 (76.2)	4.4 (2.9)	
Oct	8/11 (72.7)	11/11 (100.0)	19/22 (86.4)	4.3 (2.4)	2.4 (1.4)
Dec	3/7 (42.9)	4/13 (30.8)	7/20 (35.0)	9.7 (2.1)	4.0 (4.2)
Total	35/55 (63.6)†	33/69 (47.8)†	68/124 (54.8)	5.6‡ (5.7)	3.5‡ (3.4)

* No. infected/no. in sample (%).

† $\chi^2 = 2.48$; 1 df; $P = 0.139$.

‡ Mann–Whitney $U = 780$; $P = 0.0161$.

tion period (Fig. 2A–D) and for all collection periods combined (Fig. 2E). This negative relationship was not, however, significant for May/June ($b = 0$, $F_{0.05[1,31]} = 3.56$, $P > 0.05$), October ($b = 0$, $F_{0.05[1,56]} = 0.541$, $P > 0.05$), or December ($b = 0$; $F_{0.05[1,42]} = 3.63$; $P > 0.05$) (Fig. 2A, C, D). Negative correlations were significant for Aug ($b \neq 0$, $F_{0.05[1,53]} = 38.60$, $P < 0.05$) (Fig. 2B) and for all collection periods combined ($b \neq 0$, $F_{0.05[1,188]} = 68.90$, $P < 0.05$) (Fig. 2E).

Life cycle stages of *M. temperatus* by collection period

All worms observed in the February/March collection period were gravid (Table 1), and 60.6% of these worms had in excess of 25 eggs (Fig. 3). In May/June, relative frequencies of life cycle stages were quite different from those in the previous collection period (Fig. 3). Those worms with 1–25 eggs (i.e., n_{Aa} in Table 1) dominated, but nearly 30% of the worms were identified as juveniles, and <15% were individuals with >25 eggs (i.e., n_{Ab} in Table 1) (Fig. 3). In August, individuals with 1–25 eggs were clearly dominant, making up approximately 72% of the worms (juveniles and adults) for that collection period. October and December collection periods were dominated by individuals containing >25 eggs (Fig. 3).

Adult worm size by reproductive category and collection period

Those gravid adults with 1–25 eggs and >25 eggs in May/June were significantly smaller than their counterparts of August, October, and December (Fig. 4). Mean lengths of worms with 1–25 eggs were essentially the same for October and December and were significantly longer than the lengths of their 1–25-egg counterparts of May/June and August (Fig. 4). There were no significant differences in mean lengths of those *M. temperatus* individuals with >25 eggs for August, October, and December (Fig. 4). August was the only collection period where mean lengths of adult worms in the 3 reproductive categories were significantly different from each other (Fig. 4).

Discussion

Lack of gender-related differences in the prevalence of infection of *M. temperatus* from West Virginia was similar to the findings of Jackson and Beaudoin (1967) and Price and Buttner

(1982). In an earlier study of frog parasites, Fortner (1923, p. 85) stated that the "—percentage of infection between the two sexes does not differ to any great extent." Differences in newt weights was not a factor in sex-based prevalences. We compared weights of females and males (infected and uninfected) for the entire newt sample population. Females were heavier (4.06 ± 1.29 g) than males (3.86 ± 0.99 g), but these mean weights were not significantly different ($t_{0.05,122} = 0.990$; $P > 0.05$). Because prevalences for both sexes of newts were essentially the same, we pooled *M. temperatus* infection data (Table 2) so that comparisons of prevalences could be made with those of previous investigators. The combined prevalence of 54.8% for *M. temperatus* from West Virginia is comparable to the values of 50.6% in Pennsylvania (Jackson and Beaudoin, 1967) and 43.8% in New York (Fischthal, 1955). In contrast, lower prevalences of infection have been noted in North Carolina (25.9–31.8%) by Rankin (1937) and Mann (1932), in Virginia (20.8%) by Russell (1951), and in Massachusetts (16%) by Rankin (1945). Price and Buttner (1982) observed a prevalence of 2.6% for *M. rankini* (=*M. temperatus*) in *N. v. louisianensis* from 2 ponds in Illinois.

Prevalence of *M. temperatus* varies with season of newt collection. Rankin (1937) found that prevalences of amphistome infection in newts were highest (38–42%) in April, July, and October, decreasing to 11.1% and 25.0% (for 2 different ponds) in December. Russell (1951) recorded peak prevalences for *M. rankini* in March (51%) and July (46.4%) but found only 7.6% of the newts infected in October and none in December. The prevalence trend observed in the present study (i.e., low in February/March, increasing steadily through October, then declining in December) was reminiscent of the pattern described by Rankin (1937). Although snails were not examined for developmental stages of *M. temperatus*, it appears that rising prevalence for the May/June newt sample is a likely consequence of increased cercarial production by infected snails. This conclusion is based on the high proportion of juvenile worms recovered at necropsy during the May/June sample period (Fig. 3). This presence of juveniles, coupled with the relatively small size of adult worms (Fig. 2A), also indicates that May/June is the major recruitment period. Cannibalism of their

own larvae is an important dietary strategy for adult newts in late July and August (Burton, 1977). This adult feeding behavior may represent an additional factor contributing to increased prevalence of infection observed in the August and October collections (Table 2), because larval newts may harbor infective metacercariae. The low prevalences of February/March and December are understandable, given that cercarial production is likely low or nonexistent and that newts, being ectothermic, may exhibit reduced feeding activity during these colder months.

Observed mean intensities of 5.6 for female and 3.5 for male newts are significantly different (Table 2). Although no other published studies record mean intensities of *M. temperatus* by host sex, means observed in the present study do not appear unusual when compared with means of 4.2 noted by Mann (1932), 4.0 noted by Price and Buttner (1982), and 3.7 noted by Russell (1951).

The relationship between numbers of *M. temperatus* and size of host has not been reported. Our data suggest a positive correlation between worm numbers and host weight (Fig. 1F). However, the strength of the correlation varied inconsistently with time of collection. Intensity of digenean infection in other amphibians as a function of host size has been recently reported, also with mixed outcomes. Wetzel and Esch (1996) found no significant correlation between total numbers of *Halipegus occidualis* and snout–vent length (SVL) of *Rana clamitans,* but numbers of *H. eccentricus* were positively and significantly correlated with host SVL.

Mean lengths of *M. temperatus* individuals decreased as a function of their total numbers in a host for every collection period and overall. These negative correlations were not always significant, but their consistency coupled with the significance of the overall regression (Fig. 2E) strongly suggests a density-dependent relationship. Because there is a finite amount of space and nutrients available in the large intestines of newts, intraspecific competition for these finite resources offers a plausible explanation for density dependence. Two alternative mechanisms for density dependence, such as parasite-induced host mortality and parasite fecundity, were considered in the present study. There was no evidence of gross pathology in the large intestines of infected newts, and the health of infected

newts (if body weight can be viewed as a measure of host health) did not appear to be compromised; weights of infected newts were either comparable to their uninfected counterparts (in females) or significantly greater (in males). The question of a density-dependent effect on *M. temperatus* fecundity arose because Goater (1992) argued convincingly that high densities of the nematode *Rhabdias bufonis* (in *Bufo bufo*) were associated with a decline in worm fecundity. Density-dependent effects on fecundity of *M. temperatus* have not been addressed by previous workers nor could such effects be evaluated definitively in the present work. We have, however, some intriguing empirical data. For example, in the December collection period 4 newts harbored relatively dense *M. temperatus* populations of 8, 9, 10, and 13 worms, respectively. In 1 newt, all 8 worms were in the highest reproductive category (i.e., >25 eggs), whereas 11 of 13 worms in another newt were in the >25 egg reproductive category (1 worm was nongravid and 1 worm was lost). This observation, although limited, cautions against arguing that density dependence inhibits fecundity in *M. temperatus* populations. The newt with 10 worms appeared to represent a recent infection rather than an inhibition of fecundity; 8 of the 10 worms were nongravid with the remaining 2 worms in the 1–25-egg reproductive category. In the newt with 9 worms, 7 worms were in the 1–25-egg reproductive category and 2 worms were in the >25-egg category. This last example, however, was complicated by the fact that 3 worms in the 1–25-egg category were quite large (Fig. 2D) and may have released many of their eggs. The last example also illuminates the drawbacks of evaluating worms in natural populations, e.g., not knowing the time of initial infection(s) or exposure dose(s) as compared with assessing the growth and sexual maturation of worms monitored under laboratory conditions.

In summary, overall and seasonal prevalence of *M. temperatus* in newts from West Virginia is relatively high and is comparable to infection patterns in Pennsylvania and New York. Mean intensity levels are similar to those of previous studies. This study is the first to provide information on amphistome numbers as a function of host size at different times of the year and an evaluation of the relationship between size of amphistomes as a function of their total numbers (i.e., density dependence) and mean size of

worms by season. Studies of a similar nature in different geographic regions should be encouraged.

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